

BIOETHANOL PRODUCTION USING OIL PALM TRUNK SAP (OPTS) BY *KLUYVEROMYCES* *MARXIANUS* – EFFECT OF Ph AND TEMPERATURE

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Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

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JANUARY 2014

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ABSTRACT

This paper presents the kinetic study of bioethanol production using Oil Palm Trunk Sap (OPTS) by *Kluyveromyces marxianus*. Bioethanol have been discovered as the substitute of petrol that is biodegradable and less toxic than fossil fuels. Oil palm trunk can be used to produce bioethanol from its sap because it contains high glucose and other types of sugars. This study is focus on studying the kinetic parameters of bioethanol production from oil palm trunk sap (OPTS) using *Kluyveromyces marxianus*. By analyzing specific growth rate, sugar consumption rate and ethanol production rate the kinetic parameters can be determined. The analytical techniques for bioethanol production and substrate consumption were monitored by high-performance liquid chromatography (HPLC) and cell dry weight (CDW) for growth profile. In this study, pH and temperature were varied to study the effect of different pH (3, 4, 5, 6, and 7) and temperature (25°C, 30°C, 35°C, 40°C, and 45°C). The highest ethanol concentration was produced at pH 5 which is 26.75 g/L and at temperature 35°C ethanol concentration production was 45.06 g/L.

Keywords: *K. marxianus*, Bioethanol production, oil palm trunk sap (OPTS), Kinetic parameters

ABSTRAK

Kertas kerja ini membentangkan mengenai penghasilan bioethanol menggunakan cairan batang kelapa sawit oleh *Kluyveromyces marxianus*. Bioethanol telah ditemui sebagai pengganti kepada petrol yang mesra alam dan kurang toksik berbanding bahan bakar fosil. Cairan batang kelapa sawit boleh digunakan untuk menghasilkan bioethanol kerana ia mengandungi kandungan glukosa yang tinggi dan jenis gula yang lain. Kajian ini memfokuskan kepada kinetik parameter terhadap penghasilan bioethanol daripada cairan batang kelapa sawit oleh *Kluyveromyces marxianus*. Dengan menganalisis kadar pertumbuhan spesifik, kadar penggunaan gula dan kadar penghasilan ethanol. Teknik analisis untuk penghasilan bioethanol dan penggunaan gula telah dipantau menggunakan kromatografi cecair prestasi tinggi (HPLC) dan berat sel kering (CDW) untuk profil pertumbuhan. Dalam kajian ini, pH dan suhu telah dipelbagaikan untuk mengkaji kesan perbezaan pH (3, 4, 5, 6, dan 7) dan suhu (25°C, 30°C, 35°C, 40°C, dan 45°C). penghasilan kepekatan ethanol yang tertinggi telah terhasil di pH 5 (26.75 g/L) dan di suhu 35°C (45.06 g/L).

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LIST OF ABBREVIATIONS

μ	– Specific growth rate
h	– Hour
no.	– Number
HPLC	– High Performance Liquid Chromatography
OD	– Optical Density
CDW	– Cell dry weight
OPTS	– Oil palm trunk sap
rpm	– rotation or revolution per minutes
rs	– sugar consumption
rp	– bioethanol production
v/v	– volume per volume
w/v	– weight per volume

CHAPTER 1

INTRODUCTION

1.1 Background study

Bioethanol is the biotechnology-based production as the substitute of fuels due to the exhaustion of fossil fuels and the increase in their price. It is the alternative to replace the fossil fuels that could reduce vehicles carbon dioxide (CO₂) by 90 %. Bioethanol is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from primary feedstock and agricultural crops. These sources are grown specifically for energy use which includes corn, maize and wheat crops, waste straw, willow and poplar trees. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemicellulose and lignin. These carbon sources can be converted to bioethanol by various microorganisms.

The economics of ethanol production by fermentation is influenced by cost of the raw materials, which accounts for more than half of the production cost. In recent years bioethanol production has been produced from oil palm trunk sap. Oil palm trunk has become one of the highest production crops in the world. There is no available method to utilize felled oil palm trunks except in plywood factories. As stated by Murata *et al.*, (2012), there are only small percentages of the felled trunks use for plywood production, but nearly all of the felled trunks are discarded. Oil palm trunk is the agricultural waste that contain large amount of sugars in its sap such as glucose and sucrose. These sugars can be converted easily to ethanol and lactic acid, thus the trunk was reported to be a potential significant resource for the production of fuel ethanol, biochemical and bioplastics (Murata *et al.*, 2012). Oil palm sap was reported to contain approximately 11% sugars with sucrose as a major component accounting for approximately 90% of total sugar (Yamada *et al.*, 2010).

The production of bioethanol from OPTS usually using *Saccharomyces cerevisiae* as the microorganism. This is because this yeast strain can produce a high concentration of ethanol and it is preferred for most ethanol fermentations (Ngoh *et al.*, 2009). In this study *Kluyveromyces marxianus* is use as the microorganism to produce bioethanol from OPTS. *Kluyveromyces marxianus* is a thermotolerant yeast that shows considerable growth in the temperature range between 25°C and 45°C, while *Saccharomyces cerevisiae* did not grow at 45°C (Matsuzaki *et al.*, 2012). Since there is no data or study have been done on bioethanol production from OPTS using *K. marxianus*, this study will use OPTS as the substrate and *K. marxianus* as the microorganism. The kinetics of ethanol production using *K. marxianus* was also studied.

1.2 Motivation

Production of bioethanol from oil palm trunk sap (OPTS) has developed from time to time. The development of bioethanol production using OPTS as substrate is due to contain of high glucose content in the sap. There is no previous research has been done for bioethanol production from substrate OPTS using *Kluyveromyces marxianus* (*K. marxianus*). Therefore, it is motivated to investigate the effect of several factors in influencing bioethanol production.

1.3 Objective

The objective of this study is to determine the effect of pH and temperature in the production of bioethanol from oil palm trunk sap (OPTS) using wild strain *K. marxianus*.

1.4 Scope of study

This study investigated the effect of temperature from 25°C to 45°C and pH factor from 3 to 7 during fermentation that affecting the bioethanol production using *K.*

marxianus and OPTS. The time series of growth and bioethanol production were monitored by cell OD and cell dry weight and high performance liquid chromatography (HPLC) respectively. HPLC was also need to monitor the sugars consumption. The specific growth rate (μ) and the kinetic parameters such as glucose consumption rate (r_s) and bioethanol production rate (r_p) were determined.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This study is to investigate the kinetic parameters towards the bioethanol production using oil palm trunk sap (OPTS) by *Kluyveromyces marxianus*. Previous studies have shown that most bioethanol production was using agricultural waste and primary feedstock such as corn, sugarcane, and wheat. Using primary feedstock as the main substrate in the industrial production will compete with the source to produce food stock. Currently OPTS have been found to be useful in bioethanol production. Most of the studies using *Saccharomyces cerevisiae* as the microorganism since yeast can produce more bioethanol in sugar fermentation. There is lack of information study on *K. marxianus* as the microorganism for bioethanol production using OPTS. Other than that, *K. marxianus* has been found as the thermotolerant yeast that can produce high production of bioethanol at the higher temperature until 47°C compared to *Saccharomyces cerevisiae*.

2.2 Bioethanol production

Nowadays bioethanol production has been developed to be the substitute fuels. Dodic' *et al.*, (2012) stated that bioethanol is a modern energy source, which represents a significant replacement of liquid fossil fuels. It is necessary to consider and identify which process or combination of processes for bioethanol production gives the best results from the technological, economic and ecological aspect.

Bioethanol is one of the renewable sources for the fuels nowadays. It has been widely produced due to the high demand in population and industrialization. As stated by Sarkar *et al.*, (2012), the world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used for the production of fuel, electricity and other goods. In this scenario, renewable sources might

serve as an alternative. Bioethanol has been receiving widespread interest at the international, national and regional levels. The global market for bioethanol has entered a phase of rapid, transitional growth. Many countries around the world are shifting their focus toward renewable sources for power production because of depleting crude oil reserves. The trend is extending to transport fuel as well. Recently, the focus on renewable biofuels in Malaysia is restricted to biodiesel and bioethanol only. Most liquid fuel in Malaysia are utilized in transportation sector, that is why Malaysia need renewable energy to substitute fuel. The ethanol derived from biomass, or second-generation bioethanol (SGB), offers greater promise in replacing fossil fuels than bioethanol that was derived from edible sources, or first-generation bioethanol (FGB), because SGB does not compete with the human food supply (Tye *et al.*, 2011).

Besides solar energy, the other renewable energies are solid waste, mini hydro, biogas and biomass. The growth of different types of renewable energy resources from 2011 to 2030 is shown in Figure 2.1. From the figure, we can see that besides solar energy, the growth of biomass also increases every year. Malaysia has a significant amount of agriculture activities; thus, biomass can be a very promising alternative source of renewable energy (Tye *et al.*, 2011). Bioethanol can be produced from the fermentation of raw materials that has sugar in it to be metabolized to bioethanol. Yamada *et al.* (2010), investigated the possible ethanol yield from sap of old trunk and calculated it to be approximately 9000 L/m², which exceeds that of sugar cane juice. Other than that Kosugi *et al.* (2010) determined the amount of ethanol produced from OPTS is correspond to 94.2% of the theoretical yield calculated based on consumption of glucose, sucrose, fructose, and galactose. By this finding, unlike sugar cane, bioethanol production using felled OPT will not conflict with food usage and has a great potential as a feedstock for bioethanol.

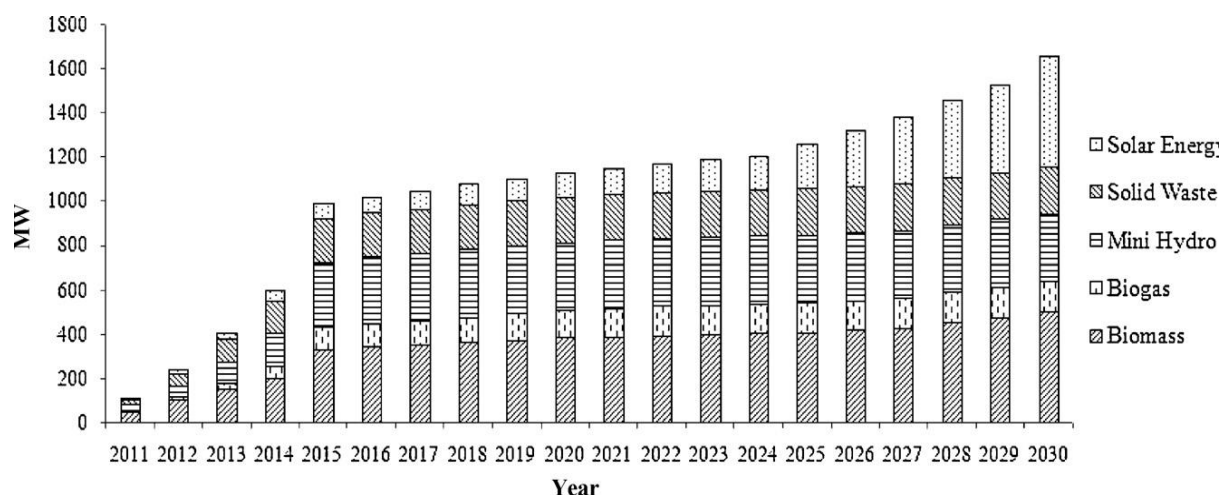


Figure 2.1: The growth of different type renewable energy resources from 2011 to 2030.

From the previous research it has been shown that bioethanol is the most important renewable source in the future. The increasing demand of the fuel will increase the demand of the bioethanol as it is the renewable energy that can obtain from the biomass. The most economical way to produce bioethanol by fermentation.

2.3 Oil palm trunk sap (OPTS) as the substrate

Malaysia is the tropical country that widely planted oil palm tree for its oil. As the oil palm is a tropical palm tree, it can be cultivated easily in Malaysia. The oil palm tree in Malaysia originates from West Africa, where it grows wild. It was later developed into an agricultural crop. Because oil palm is a high yielding crop, it can produce, on average, about 4-5 ton of oil/year. It has been forecasted that, in years to come, the demand will be higher with increasing world demand in oils and fats. It is already very profitable to invest in the oil palm industry in Malaysia, even just using existing technology (Tye *et al.*, 2011). As stated by Yamada *et al.* (2010), the oil is mainly used for food, raw material for various products such as detergents and cosmetic.

Oil palm trees are replanted at an interval of approximately 25 years because of decreased oil productivity, so the felled trunks are the enormous amount of biomass resources in the palm oil produces. The oil palm trunks usually utilized for plywood manufacturing and the inner part is discarded because of its weak properties for

manufacturing of the plywood. When replanting the oil palm trees, the old trunks are cut and most of them are discarded or burnt. This will produce pollution and abundant of biomass will produce. Therefore, the most efficient ways needed for utilizing oil palm trunks for the ideal oil palm plantation and sustainable palm oil industry (Yamada *et al.*, 2010).

From previous study, in order to utilize the old palm trunks felled for replanting, especially the inner part, attempt has been done to produce bioethanol and the material for bio-plastic from felled trunks (Kosugi *et al.*, 2010). The study focused on sugars in the sap of the felled trunk and observed a large quantity of high glucose content sap in the trunk. The water content of the trunk is surprisingly high at a mass fraction of 70-80%, which is much higher than that of freshly harvested wood species around 50-60% (Murata *et al.* 2012). Their research found that the major sugar in the sap from oil palm is glucose and the minor sugar components in the sap medium such as sucrose, fructose and galactose initially found at 4.2 g/L, 2.6 g/L and 0.6 g/L respectively. Because of the large amount of glucose in the sap, it can be a good feedstock for the bioethanol production.

Yamada *et al.* (2010), has investigated that the free sugar content in the sap is at the maximum in 30 to 60 days of storage after logging. Therefore the sap should be squeezed during this period to obtain the highest sugar concentration for the production of bioethanol. Studies have shown that oil palm will absorb carbon dioxide (CO₂) and return oxygen (O₂) to the atmosphere more than others plant, thus the conversion of oil palm biomass into second-generation bioethanol to be used as transport fuel can further reduce the emission of CO₂ and conservation of environment can be achieved (Tye *et al.*, 2011).

Other research also state that sugar cane has sufficient organic nutrients and minerals that make it more suitable for the microorganism especially *Saccharomyces cerevisiae* for ethanol production (Limtong *et al.*, 2007).

From the research that has been done, felled oil palm trunk was the most abundant crops in Malaysia. It also have been proved to contain highest sugar concentrations, such as glucose, sucrose, fructose and galactose, which can be used for production of bioethanol. So, oil palm trunk sap was the most suitable substrate for the

production of bioethanol rather than sugar cane as it prevents the conflict with food usage and has a great potential as a feedstock for bioethanol.

2.4 *Kluyveromyces marxianus* as the microorganism

Ethanol fermentation is a biological process in which organic chemical is converted by microorganism to simpler compounds, such as sugars. These fermentable compounds are then fermented by microorganisms to produce ethanol and CO₂. During the whole process of ethanol fermentation, there are mainly two parts for microorganisms. One is for the microorganisms which convert fermentable substrates into ethanol, and the other is to produce the enzyme to catalyze chemical reactions that hydrolyzed the complicated substrates into simpler compounds (Lin and Tanaka, 2006). Furthermore, the lack of industrially suitable microorganisms for converting biomass into fuel ethanol has traditionally been cited as a major technical roadblock to developing a bioethanol industry.

Matsuzaki et al. (2012), stated that *K. marxianus* is a thermotolerant yeast that shows a considerable growth in the temperature range between 25°C and 45°C. Thermotolerant microorganism is the efficient way for the ethanol fermentation at high temperature in tropical countries, where average day-time temperatures are usually high throughout the year. To achieve high temperature fermentation, it is necessary to use an efficient yeast strain that can tolerate high temperature (Eidpum *et al.*, 2012).

Eidpum *et al.* (2012) investigated the high temperature ethanol fermentation by comparing between *K. marxianus* and *S. cerevisiae*. They found that *S. cerevisiae* more effective to produce bioethanol in operating temperature range of 33°C to 37°C while *K. marxianus* more effective to produce bioethanol at high temperatures range 40°C to 45°C.

Malaysia is one of the tropical country in the world that average day time temperatures are usually high throughout the year. To effectively utilize the condition of the environment, *K. marxianus* may be more efficient microorganism used for the bioethanol fermentation as it can growth at high temperature.

2.5 Factors affect the bioethanol production

There are several factors that affect the bioethanol production such as pH, temperature and substrate concentration. The effects of these factors depend on the characteristics of the microorganism that was used in the production of bioethanol. In this study, two kinetic parameters, temperatures and pH, were investigated.

2.5.1 Effect of pH

pH is one of the important kinetic parameters that affect the microorganism's growth and production rates. Yeast was found to prefer acidic condition for the optimum growth and production. Lin and Tanaka (2006) found that most yeast strain can grow in the pH range 4.5 to 6.

Manikandan *et al.*, (2008) investigated the effect of pH on bioethanol production from banana peel waste using *S. cerevisiae*. They conducting the experiment in the pH range of 3.5 to 5.5 and the results show that highest ethanol production was at pH 4.7 (9.2 g/L). In another study, Manikandan *et al.* (2010) also using *S. cerevisiae* for the investigation of ethanol production but they used corn flour as the substrate instead of banana peel. This time they found that the optimum pH for the ethanol production was 5.5 with the production of 49.037 g/L. Their results revealed that different substrates will affect the optimum pH for ethanol production even though similar microorganism was used.

K. marxianus is a type of yeast that have similar pH range to grow as *S. cerevisiae*. Limtong *et al.* (2007), had investigated the effect of pH on bioethanol production from sugar cane juice using *K. marxianus*. They studied the pH range of 4 to 5.5 and found at pH 5 and pH 5.5, the highest production of bioethanol (8.7% (w/v) and 8.5% (w/v) respectively) could be obtained. Most of the other study that used *K. marxianus* as the misroorganism for bioethanol production used constant pH 5 for the fermentation. Similarly, Eiadpum *et al.* (2012) they using blackstrap molasses and cane juice as the main substrate for ethanol fermentation and they also set constant the pH of fermentation at 5 with though different culture temperature were used.

There is not much investigation for the pH study of *K. marxianus* was done. Since it is a type of yeast, it is predicted that the optimum pH will lie between 4.5 to 5.5 in acidic region.

2.5.2 Effect of temperature

Temperature plays a main role in the fermentation that depends on the microorganism type. Microorganism have been classified according to the optimum temperature for growth, psychrophiles optimum at < 20°C, mesophiles optimum from 20°C to 50°C and thermophiles optimum at > 50°C.

Manikandan *et al.* (2010) studied the ethanol production by *S. cerevisiae* using corn flour as the substrate at different temperature from 28°C to 36°C. They found that the optimum temperature for the ethanol production was at 30°C (63.04 g/L). In another study, Kosugi *et al.* (2010) using similar microorganism but in oil palm trunk sap substrate. They found that the fermentation was almost complete after 12 h that reached stationary phase and glucose was thoroughly consumed after 24 h. The fermentation was conducted at 30°C and produce 30 g/L ethanol concentration. Yeast was the microorganism that have optimum grow at 30°C to produce ethanol.

Oda *et al.*, (2010) investigated the ethanol fermentation using sugar beet juice and crude cheese whey using *K. marxianus* at constant pH 5 for fermentation. Their results showed that ethanol was produced at slower rate at 30°C (70 mg/ml) if compared to temperature at 33°C (90 mg/ml) to 37°C (100 mg/ml). Eiadpum *et al.* (2012) studied the co-culture of *K. marxianus* and *S. cerevisiae* for their abilities to improve the production and stability of ethanol fermentation. From this study, they found that *K. marxianus* was able to produce ethanol at high temperature of 40-45°C in the sugarcane medium, while *S. cerevisiae* was more effective in producing ethanol at 33°C to 37°C. Thermotolerant microorganism was efficient to produce high bioethanol than the mesophile microorganism that cannot survive in the high temperature.

From the research above, it can be concluded that optimum temperature in the producing of bioethanol is very much depend on the type of the microorganism.

2.6 Kinetic study on effect of pH and temperature

Kinetics is the study of changes in a physical or chemical system. Evaluation of kinetic parameters is essential for process scale-up. This research's objective was to investigate the kinetic study of bioethanol production from oil palm trunk sap (OPTS) by wild strain *K. marxianus*. The parameters that were investigated in this study consist of specific growth rate (μ), ethanol production rate (r_p) and substrate consumption rate (r_s).

Dodic' *et al.* (2012) studied the kinetic on ethanol production from sugar beet raw juice. They stated that the kinetics of growth could be quantified using Monod's equation and based on values of biomass, bioethanol and fermentable sugars concentration measured every 2 h throughout the process, the calculations for biomass production rate (r_x), fermentable sugars consumption rate (r_s), bioethanol production rate (r_p) and specific growth rate μ were made. From their result the fermentable sugars consumption rate and bioethanol production rate reached their maximum at 10 h (7.19 g/l.h and 4.17 g/l.h, respectively).

Limtong *et al.* (2007) studied the production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *K. marxianus*. They found that sugar utilization further confirmed the low ethanol production and yeast cell growth. Ehen the experiment was conducted in the condition where the highest ethanol production and growth were obtained, gave the lowest sugar concentration remaining at the end of fermentation (7.05% w/v). However, under condition with the lowest ethanol production and growth, the highest concentration of remaining sugar (10.35% w/v) was observed. This show that when highest remaining sugar at the end of fermentation was observed, the sugar consumption for ethanol production was lower. From these study, the highest consumption rate was at pH 5 an temperature 35°C which was the highest growth rate and the highest ethanol formation rate at the similar condition.

CHAPTER 3

METHODOLOGY

3.1 Raw Materials and Chemical

The raw material in this research was oil palm trunk sap (OPTS) that was collected from Jengka, Pahang. Pure culture of wild type *Kluyveromyces marxianus* was used in this study. Table 3.1 shows the chemicals that were used in this study.

Table 3.1: Chemical used in this study

No.	Chemical	Brand
1	Bacto-Tryptone	OXOID
2	Yeast extract	OXOID
3	Sodium chloride (NaCl)	Sigma Aldrich
4	Agar powder	Sigma Aldrich
5	Sodium hydroxide (NaOH)	Merck
6	Hydrochloric acid (HCl)	Merck
7	Standard ethanol (HPLC solution)	Merck
8	Standard glucose (HPLC solution)	Sigma Aldrich
9	Standard sucrose (HPLC solution)	Sigma Aldrich
10	Standard fructose (HPLC solution)	Sigma Aldrich

3.2 Medium preparation

Nutrient agar, nutrient broth and oil palm trunk sap (OPTS) were used as medium in this study. For solid medium, the composition of agar containing 20 g/L agar, 10 g/L peptone, 5 g/L yeast extract and 5 g/L of glucose according to Yeast extract, Peptone & Glucose (YPD medium) ingredients. The nutrient agar was being autoclaved (Hirayama HV110 Hiclave) at 121°C for 15 minutes followed by cooling. After cooling, the nutrient agar was poured into the sterilized petri plate. The plates were left undisturbed until the agar solidifies (Liu *et al.*, 2010). Nutrient broth was prepared by mixing the same ingredients of YPD medium as in nutrient agar but excluding agar powder.

Oil palm trunk obtained from oil palm plantation. The trunk was cut about 7 cm thick and the inner part of the disk was taken. The sap was collected by pressing the disk. This sap was centrifuged at 6,000 rpm (Eppendorf Centrifuge 5810R) for 15 minutes to remove the debris that containing in the sap and the supernatant was stored at -20°C before use.

3.3 Microorganism preparation

K. marxianus was cultured in the nutrient agar to get the single colony using streaking method. The agar plate was incubated in the incubator Memmert model 100 - 800 upside down at 30°C for 15-20 hours to prevent the vapor mix with the bacteria. To maintain the pure culture supply continuity for longer time, the strain was cultured in the glycerol stock at -80°C.

3.4 Inoculums preparation

After *K. marxianus* has been grown into a single colony on nutrient agar, 2 loops of the single colony were transferred into 100 ml sterile nutrient broth in 250 ml conical flask. The strain was incubated for 16 hours at 30°C and 150 rpm on rotary shaker until the initial optical density (OD) achieved was 1.5. After 16 hours, the cells were harvested by centrifuging for 10 minutes at 10,000 rpm. Then, cell washing was done to prevent the carryover of spent medium or any waste. The cells were then resuspended with OPTS medium to start the fermentation.

3.5 Fermentation Preparation

Fermentation studies were performed in 250 ml conical flask. 10 % (v/v) of inoculums suspension was transferred to fermentation flask that containing 90 % (v/v) of OPTS medium. Before that, the sap was sterilized in the autoclave at 121°C for 20 min. The medium was incubated on rotary shaker at 150 rpm. Fermentation samples 2 ml were collected and centrifuged to remove cells at every 2 hours for 24 hours. The supernatant was filtered with 0.22 μ m Nylon filter membrane to remove any solid particles. All the procedures were repeated at 25°C, 30°C, 35°C, 40°C and 45°C with the fix pH of 5.5 and at a fix temperature of 30°C with varying of pH at 3, 4, 5, 6 and 7.

3.6 Analytical Method

The growth profile of the *K. marxianus* was monitored by checking the optical density every 2 hours for 24 hours. The absorbance of the sample was measured with a UV-VIS spectrophotometer (HITACHI 1800) at 600 nm. The cell dry weight of the samples was determined by analytical balance.

Sugar components or substrate consumption and ethanol concentration were determined using *high-performance liquid chromatography* (HPLC) equipped with an automatic sampler/injector (Agilent 1200 Series). The column type is REZEX ROA-Organic Acid with the mobile phase of 0.005 N sulphuric acid. The column temperature was controlled at 30°C. The solvent flow rate was maintained at 0.5 mL/min. The peak was detected using refractive index detector (RID). The range for calibration curve for sugar and ethanol were 10 g/L, 20 g/L, 30 g/L, 40 g/L and 50 g/L. These calibration was used to identify the amount of sugar consumption in OPTS and the ethanol concentration that was produced.

The kinetic parameters that were analyzed include μ , r_p and r_s . μ is the specific growth rate of the microorganisms. The specific growth rate can be obtained from the slope of $\ln X/X_0$ cell dry weight of the microorganism at log phase of profile.

Volumetric rate of substrate consumption, r_s ,

$$r_s = -\frac{dS}{dt} = -\frac{\mu X}{Y_{X/S}} = q_s X$$

The value of q_s can be obtained from the experimental data through the slope ds/dt that in the plot of total sugar concentration versus time. From the value of slope ds/dt , the value of $q_s \times X$ was obtained and equal to the rate of substrate consumption r_s .

The specific rate of substrate consumption, q_s

$$q_s = -\frac{1}{X} \frac{dS}{dt}$$

The volumetric rate of product formation, r_p

$$r_p = \frac{dP}{dt} = q_p X$$

The value of q_p can be obtained from the experimental data through the slope dp/dt that was in the plot of ethanol concentration versus time. From the value of slope dp/dt , the value of $q_p \times X$ was obtained and equal to the rate of product formation, r_p .

The specific rate of product formation, q_p

$$q_p = \frac{1}{X} \frac{dP}{dt}$$

CHAPTER 4

RESULT & DISCUSSION

4.1 Effect of pH and temperature on growth

The effect of kinetic parameters which is temperature and pH towards production of bioethanol were studied using oil palm trunk sap (OPTS) as the substrate and wild type *K. marxianus* as the microorganism. The cell dry weight from the fermentation using conventional nutrient broth was collected as the control for the growth curve of *K. marxianus* as shown in figure 4.1. This growth profile was act as the starter for the inoculums in the large scale with the same optical density and same concentration of microorganism. The cell dry weight was collected every 2 hour interval for 24 hours. As stated by Shuler *et al.* (2002) it consists of lag phase, exponential phase, stationary phase and death phase. From this figure the lag phase occurs in the first 2 hour where the cell was adapting with the new environment in the medium. During this phase, cell mass may increase a little, without an increase in cell number density. The exponential phase starts after 2 hours inoculums. In this phase, the cells have adjusted to their new environment. After the adaptation period, cells multiplied rapidly and cell mass and cell number density increase exponentially with time. This is a period in which all components of cell grow at the same rate. The deceleration phase follows the exponential phase. In this phase, growth decelerates due to either depletion of one or more essential nutrients. The stationary phase starts at 16 hour, which is at the end of the deceleration phase. This is a stage when the net growth rate is zero or the growth rate is equal to the death rate. Then, the last phase is death phase that follows the stationary phase. Some cell death may start during stationary phase. At the end of stationary phase, because of either nutrient depletion or toxic product accumulation, the death phase begins. The lack of nutrient and life span of the *K. marxianus* is the factor of death cell.